

Title: GYLOCOSYLATED
HUMANIZED B-CELL SPECIFIC
ANTIBODIES
Inventor(s): Shui-on LEUNG et al.
DOCKET NO.: 018733-1359

1 / 16

		L1	KSSQSVLYSANHKNYLA	WYQQKPGQSPKLLIY	WASTRES
murine	LL2	DIQLTQSPSSLAVSAGENVTMSC	-----	-----	-----
	LL2	REIHuvK	SA-V-DR	KA	-----
REI	FRs	DIQMTQSPSSLSASVGDRVTITC	WYQQTPGKAPKLLIY	-----	-----
		L2			
		L3	HQYLSSWT	FGGGTKLEIKR	-----
murine	LL2	GVPDRFTGSGSGTDFLTISRVQVEDLAIYYC	-----	-----	-----
	LL2	REIHuvK	S-S-F-SL-P-I-T-	FGQGTKLQITR	-----
REI	FRs	GVPNSRFSGSGSGTDFRTISSLQPEDIAATYC	-----	-----	-----

FIG. 1A

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DOCKET NO.: 018733-1359

2/16

Murine EUBuVH1 EUBuVH2	FRI QVOLOESGAELSKPGASVMSCKASGYTT ---Q---VK---S---V ---VQ---VK---S---V	H7 SYLWH WILKORPGOGLEMIG -VR-A -VR-A	FRI WILKORPGOGLEMIG -VR-A -VR-A
Murine EUBuVH1 EUBuVH2	H2 YINPRNDYTHEYNONPKD ----- -----	FRI EATLTADKSSSTAYMOLSSLTSEDSAVTCAE ---I---E-TN---E---R---T-F-P--- ---I---E-TN---E---R---T-F-P---	
Murine NEWMHuVH1 NEWMHuVH2	H3 RDITYTFY -----	FRI WGOGTTLVSS ----- -----	

FIG. 1B

Title: GYLOCOSYLATED
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DOCKET NO.: 018733-1359

3/16

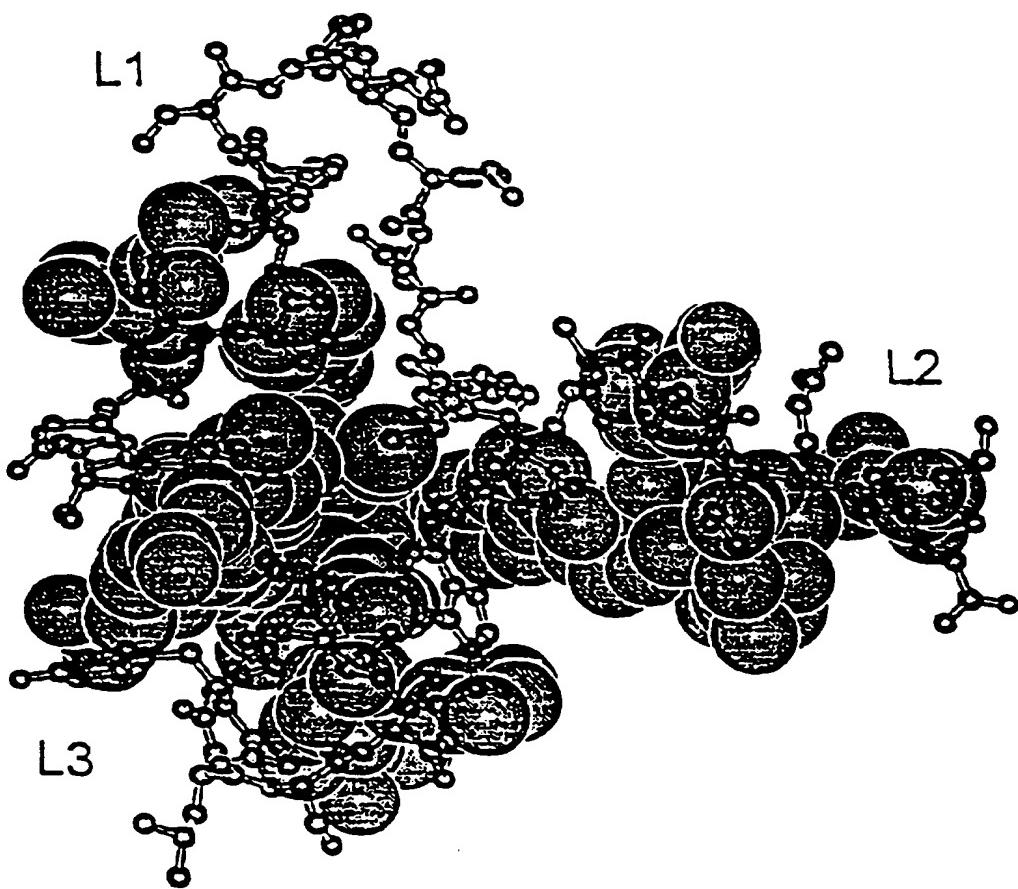


FIG. 2A

Title: GYLOCOSYLATED
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DOCKET NO.: 018733-1359

4/16

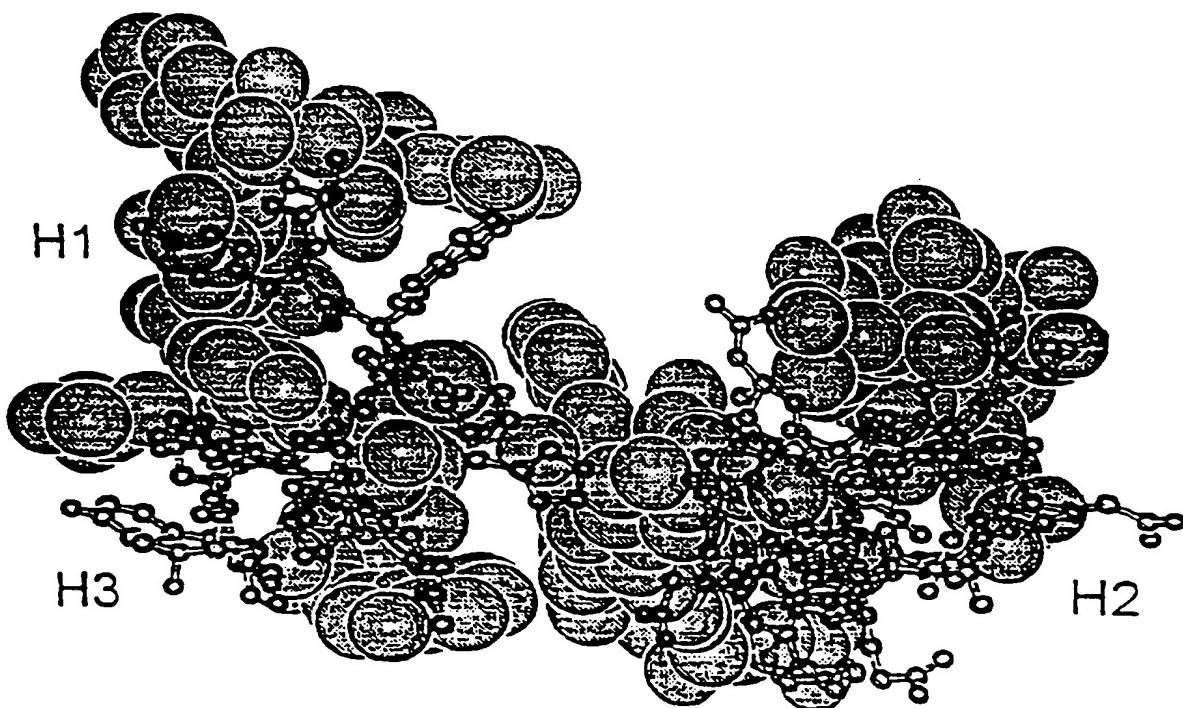


FIG. 2B

Title: GYLOCOSYLATED
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DOCKET NO.: 018733-1359

5/16

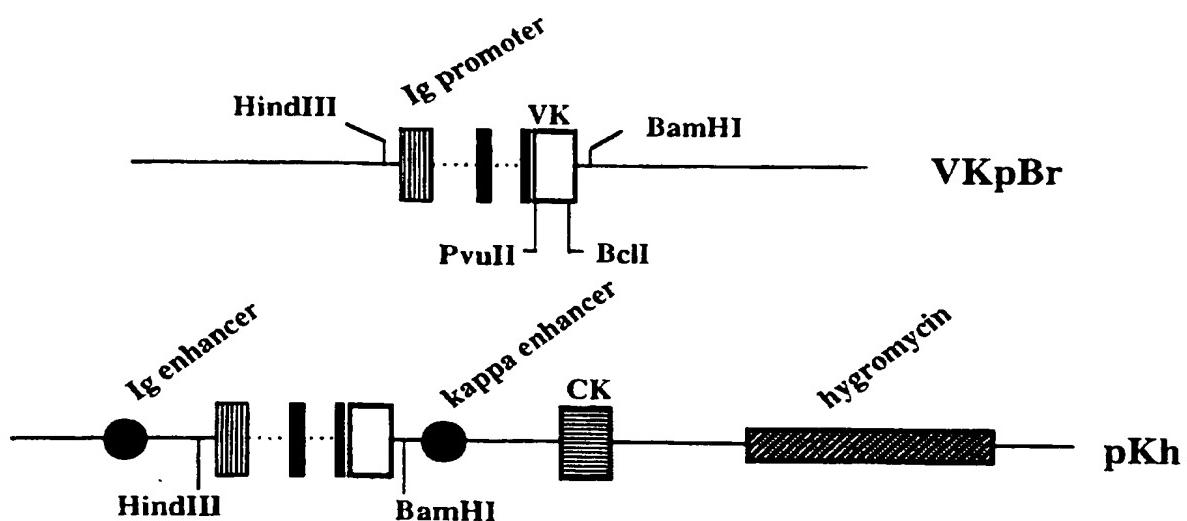


FIG. 3A

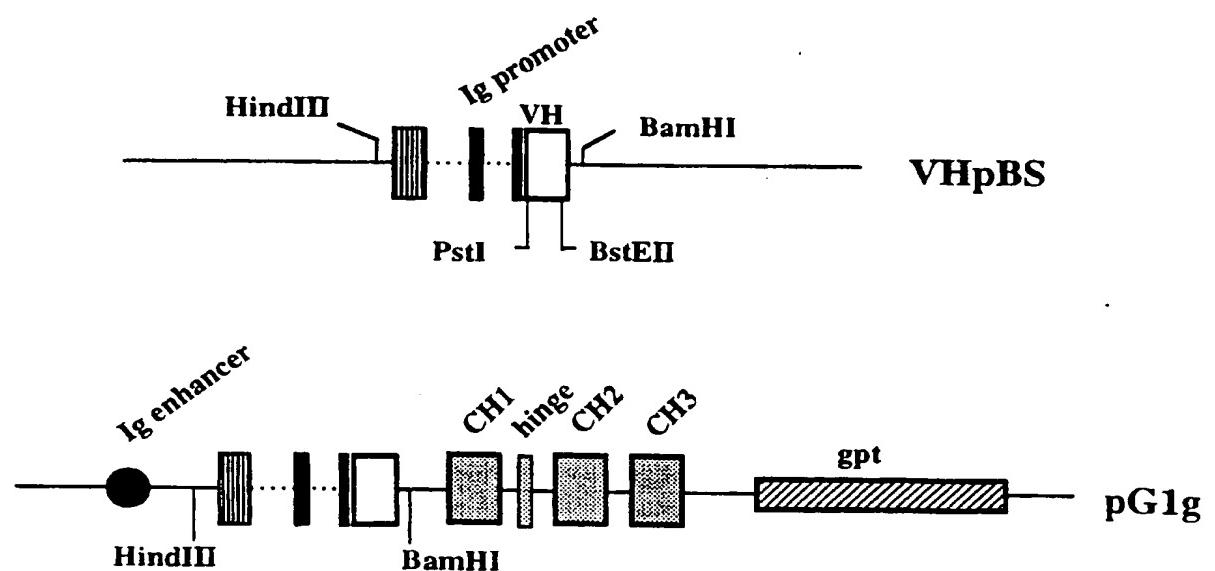


FIG. 3B

Title: GYLOCOSYLATED
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DOCKET NO.: 018733-1359

6/16

LL2 VK

SEQUENCE ID NO. 3

1	GACATTCACTGACCCAGTCTCCATCATCTCTGGCTGTCTGCAGGAGAAAACGTCACT	+ 60
	CTGTAAGTCGACTGGGTCAAGAGGTAGTAGAGAACCGACRACAGACGTCTCTTTGCAGTGA	
	D I Q L T Q S P S S L A V S A G E N V T -	
61	ATGAGCTGTAAGTCCAGTCAGTGTAACTACAGTGCATACTACAGAAGAACTACTTGGCC	+ 120
	TACTCGACATTCAAGGTCAAGTTCACAAATATGTACCGTTAGTGTCTTGATGAACCGG	
	CDR1	
	M S C K S S Q S V L Y S A N H K N Y L A -	
121	TGGTACCCAGCAGAAACCAGGGCAGTCCTAAACTGCTGATCTACTGGGCATCCACTAGG	+ 180
	ACCATGGTCTGTTGGTCCCCCTCAAGAGGATTTGACGACTAGATGACCCGTAGGTGATCC	
	CDR2	
	W Y Q Q K P G Q S P K L L I Y W A S T R -	
181	GAATCTGGTGTCCCTGATCGCTTCACAGGCAGCGGATCTGGGACAGATTTTACTCTTACC	+ 240
	CTTAGACCAACAGGGACTAGCGAAGTGTCCGTCGCCAGACCCGTCTAAATGAGAATGG	
	E S G V P D R F T G S G S G T D F T L T -	
241	ATCAGCAGAGTACAAGTTGAAGACCTGCAATTATTATGTACCAATACCTCTCTCG	+ 300
	TAGTCGTCTCATGTTCAACTCTGGACCCCTAAATAATAACAGGGTTATGGAGAGGAGC	
	CDR3	
	I S R V Q V E D L A I Y Y C H Q Y L S S -	
301	TGGACGTTGGTGGAGGGACCAAGCTGGAGATCAAACGT	339
	ACCTGCCAGCCACCTCCCTGGTTCGACCTCTAGTTCCA	
	W T F G G G T K L E I K R -	

FIG. 4A

Title: GYLOCOSYLATED
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DOCKET NO.: 018733-1359

7/16

LL2 VH

SEQUENCE ID NO. 4

CAGCTCCAGCTGCAGGAGTCAGGGGCTGAACCTGTCAAAACCTGGGGCTCAGTGAAGATG
 1 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 60
 GTCCAGGTGCGACGTCTCAGTCCCCACTTGACAGTTGGACCCGGAGTCRCTTCTAC

 Q V Q L Q E S G A E L S K P G A S V K M -

 TCCTGCAAGGCTTCTGGCTACACCTTACTAGCTACTGGCTGCACTGGATAAAACAGAGG
 61 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 120
 AGGACGTTCCGAGACCGATGTGGAAATGATCCGATGCCGACGTGACCTATTGTCTCC
 CDR1
 S C K A S G Y T F T S Y W L H W I K Q R -

 CCTGGACAGGGTCTGGAATGGATTGGATACATTAATCCTAGGAATGATTATACTGAGTAC
 121 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 180
 GGACCTGTCCCAGACCTTACCTAACCTATGTAATTAGGACTTACTAATATGACTCATG
 CDR2
 P G Q G L E W I G Y I N P R N D Y T E Y -

 AATCAGAACTTCAGGACAAGGCCACATTGACTGCAGACAAATCCTCCAGCACAGCCTAC
 181 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 240
 TTAGTCTTGAAGTTCTTCCGGTGTAACTGACGTCTGTTAGGAGGTGTCGTGTCGGATG

 N Q N F K D K A T L T A D K S S S T A Y -

 ATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCACTTACTGTGCAAGAAGGGAT
 241 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 300
 TAGCTTGACTCGTCGGACTGTAGACTCCTGAGACGTCAGATAATGACACGTTCTCCCTA

 M Q L S S L T S E D S A V Y Y C A R R D -

 ATTACTACGTTCTACTGGGCCAAGGCACCACTCTCACAGTCTCCTCG
 301 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 348
 TAATGATGCAAGATGACCCGGTCCGTGGTAGAGTGTCAAGAGGAGC
 CDR3
 I T T F Y W G Q G T T L T V S S -

FIG. 4B

Title: GYLOCOSYLATED
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ANTIBODIES
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DOCKET NO.: 018733-1359

8/16

SEQUENCE ID NO. 5

GACATTCACTGACCCAGTCTCCATCATCTCTGAGCGCCTCTGGAGATAGGGTCACT
 1 -----+-----+-----+-----+-----+-----+-----+-----+ 60
 CTGTAAGTCGACTGGTCAGAGGTAGTAGAGACTCGCGTAGACAACCTCTATCCCAGTGA

D I Q L T Q S P S S L S A S V G D R V T -

ATGAGCTGTAAGTCCAGTCAAAGTGTGTTATACAGTGCAAATCACAAGAACTACTTGGCC
 61 -----+-----+-----+-----+-----+-----+-----+-----+ 120
 TACTCGACATTCAAGGTCAGTTCAACAAAATATGTCACGTTAGTGTCTTGATGAACCGG
 CDR1
 M S C K S S Q S V L Y S A N H K N Y L A -

TGGTACCGAGCAAACCAGGGAAAGCACCTAAACTGCTGATCTACTGGCATCCACTAGG
 121 -----+-----+-----+-----+-----+-----+-----+-----+ 180
 ACCATGGTCGTCTTGGTCCCTTCGTGGATTGACGACTAGATGACCCGTAGGTGATCC
 CDR2
 W Y Q Q K P G K A P K L L I Y W A S T R -

GAATCTGGTGTCCCTTCGGATTCTCTGGCAGCGGATCTGGACAGATTTACTTTCACC
 181 -----+-----+-----+-----+-----+-----+-----+-----+ 240
 CTTAGACCACAGGGAAAGCGCTAAGAGACCGTGCCTAGACCCGTCTAAATGAAAGTGG
 E S G V P S R F S G S G S G T D F T F T -

ATCAGCTCTTCAACCAGAACATTTGCAACATATTATTGTCACCAATACCTCTCC
 241 -----+-----+-----+-----+-----+-----+-----+-----+ 300
 TAGTCGAGAGAAAGTTGGTCTTGTAAACGTTGATAATAACAGTGGTTATGGAGAGGAGC
 CDR3
 I S S L Q P E D I A T Y Y C I B Q Y L S S I -

TGGACGTTGGTGGAGGGACCAAGGTGAGATCTGCCGGTCTCCC
 301 -----+-----+-----+-----+-----+-----+-----+-----+ 345
 ACCTGCAAGCCACCTCCCTGGTTCACGTCTAGACGGCCAGAGGG
 W T I F G G G T K Y Q I C R S P -

FIG. 5A

Title: GYLOCOSYLATED
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Inventor(s): Shui-on LEUNG et al.
DOCKET NO.: 018733-1359

9/16

SEQUENCE ID NO. 6

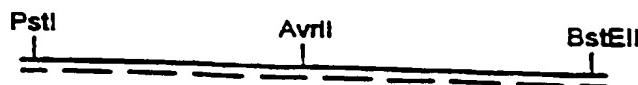
1	CAGGTCCAGCTGGTCCAATCAGGGGCTGAAGTCAGAAGAAACCTGGGTATCAGTGAAGGTC	60
	GTCCAGGTGACCGAGGTTAGTCCCCGACTTCAGTTCTTGGACCCAGTAGTCACCCAG	
	Q V Q L V Q S G A E V K K P G S S V R V -	
61	TCCTGCAAGGCTTCTGGCTACACCTTACTAGCTACTGGCTGCAGGGTCAGGCAGGCA	120
	AGGACGTTCCGAAGACCGATGTGAAATGATCGATGACCGACGTGACCCAGTCCGTCCGT	
	CDR1	
	S C K A S G Y T F T [S Y W L H] W V R Q A -	
121	CCTGGACAGGGTCTGGAATGGATTGGATACTTAATCCTAGGAATGATTATACTGAGTAC	180
	GGACCTGTCCCAGACCTAACCTATGTAATTAGGATCCTTACTAATATGACTCATG	
	CDR2	
	P G Q G L E W I G [Y I N P R N D Y T E Y] -	
181	AATCAGAACTTCAAGGACAAGGCCACAATAACTGCAGACGAATCCACCAATAACGCC	240
	TTAGTCTTGAAGTTCTGTTCCGGTTATTGACGTCTGCTTAGGTGGTTATGTCGGATG	
	[N Q N F K D] K A T I T A D E S T N T A Y -	
241	ATGGAGCTGAGCAGCCTGAGGTCTGAGGACACGGCATTATTTATTTTGCAAGAAGGGAT	300
	TACCTCGACTCGTCGGACTCCAGACTCCTGTGCCGTAAAAATAAAAACACGTTCTCCCTA	
	M E L S S L R S E D T A F Y F C A R [R D] -	
301	ATTACTACGTTCTACTGGGGCCAAGGCACCACGGTCACCGTCTCCTCG	348
	TAATGATGCAAGATGACCCGGTCCGTGGTGCCAGTGGCAGAGGAGC	
	CDR3	
	I T T F Y W G Q G T T V T V S S -	

FIG. 5B

Title: GYLOCOSYLATED
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DOCKET NO.: 018733-1359

10/16

Designed sequence for humanized LL2 VH domain:



Construction of the humanized LL2 VH domain by long oligonucleotides and PCR:

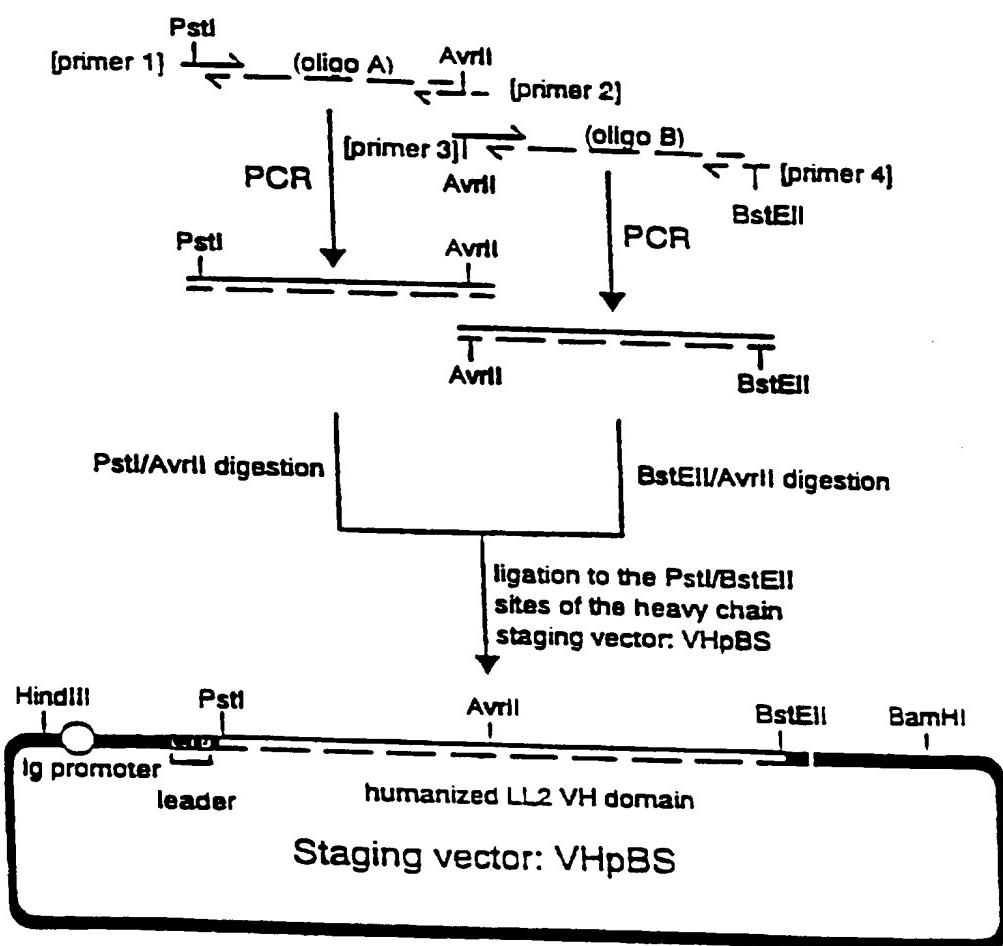


FIG. 6

Title: GYLOCOSYLATED
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ANTIBODIES
Inventor(s): Shui-on LEUNG et al.
DOCKET NO.: 018733-1359

11/16

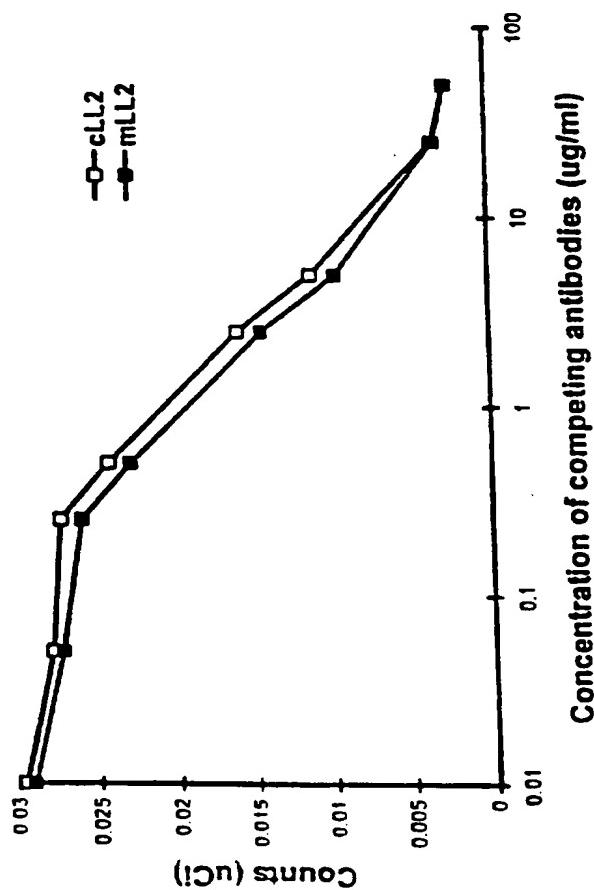


FIG. 7

12/16

FIG. 8B

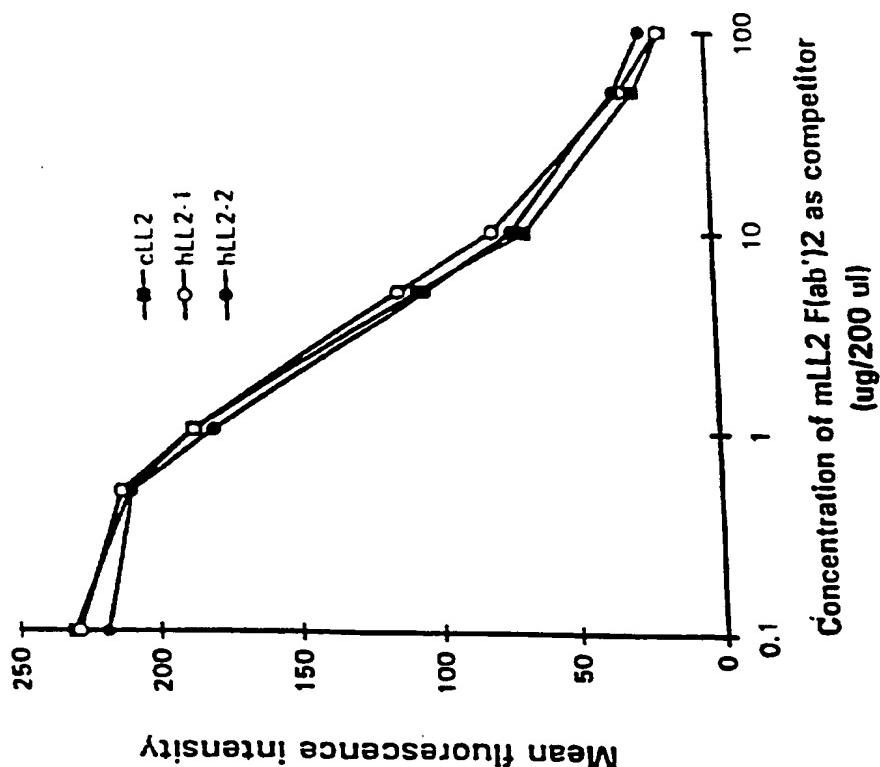
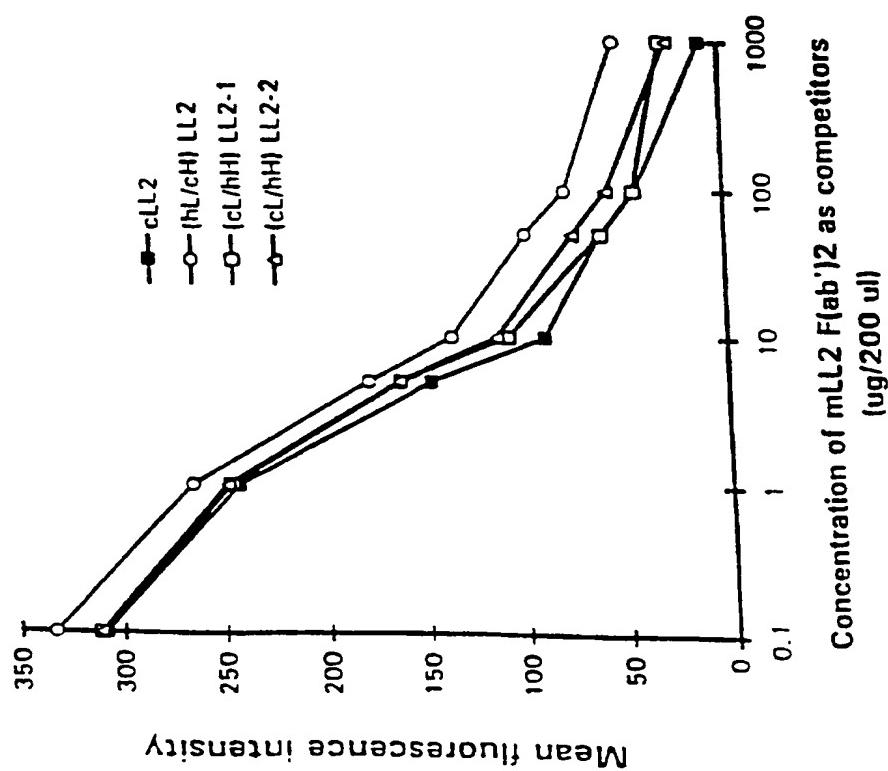


FIG. 8A



Internalization: c-LL2, h-LL2 vs. m-LL2 in Raji cells

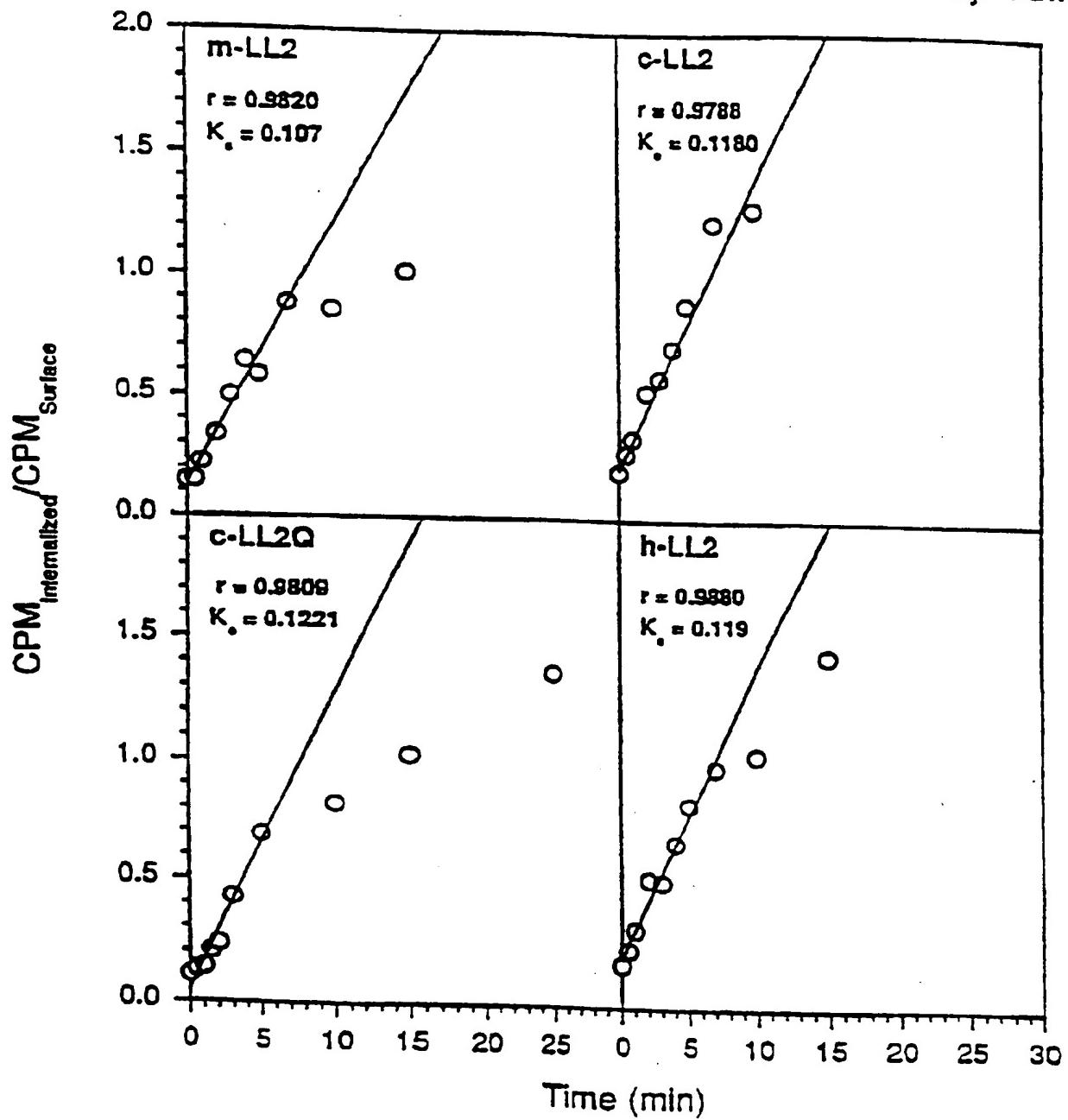
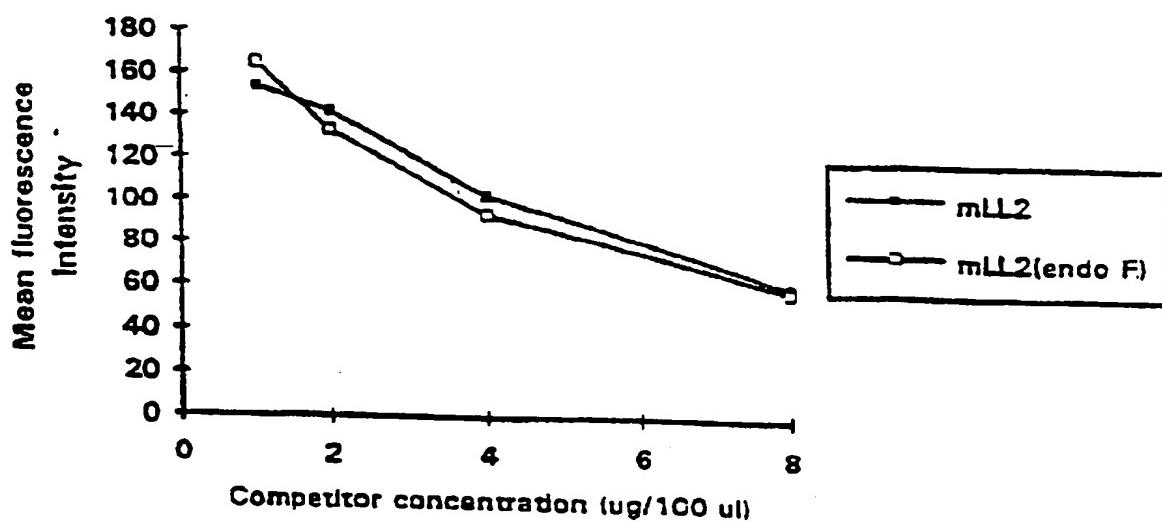


FIG. 9

Title: GYLOCOSYLATED
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ANTIBODIES
Inventor(s): Shui-on LEUNG et al.
DOCKET NO.: 018733-1359

14/16

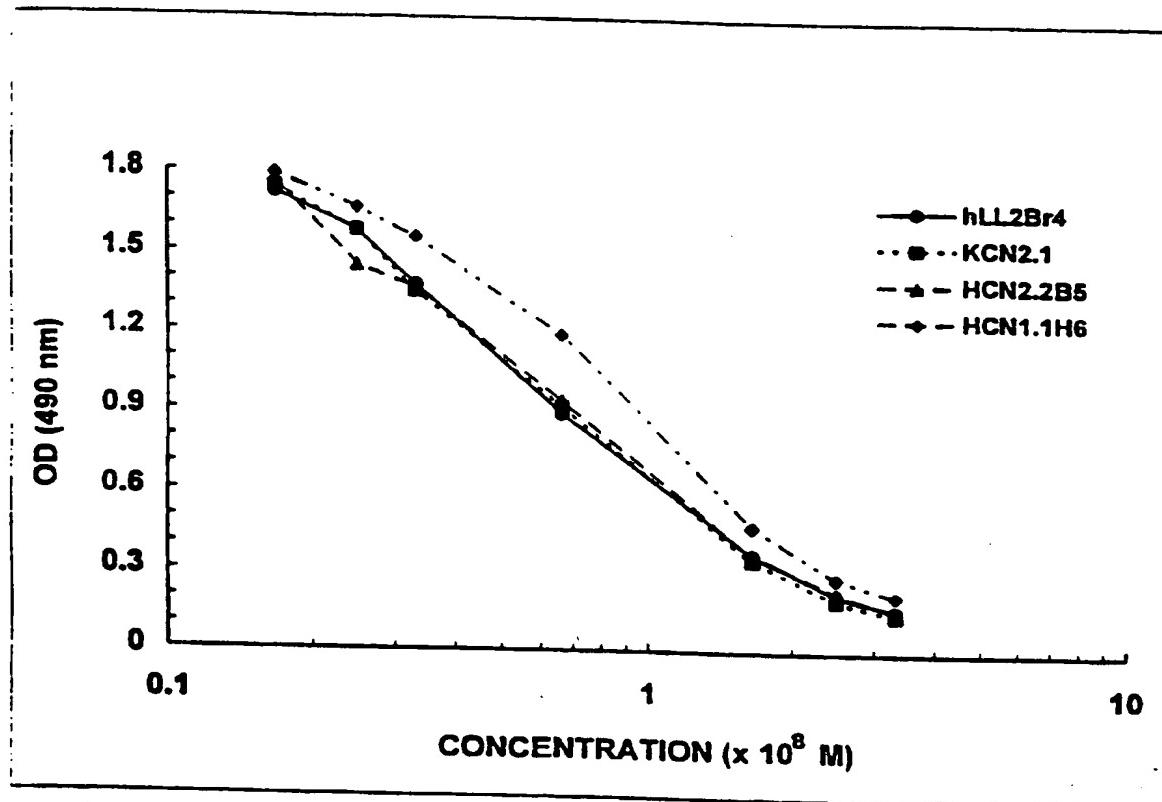


— FIG. 10 —

Title: GYLOCOSYLATED
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DOCKET NO.: 018733-1359 }

15/16

FIG. 11



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ANTIBODIES
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DOCKET NO.: 018733-1359

16/16

FIG. 12